EXHIBIT B, PAGE 1

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→ Sacrifice of mice from experiment: adjuvant effect of chemokines (hMCP-4, hMIP-3α, hTECK Peprotech) on the anti-βgal response (plasmid).
Immunizations: injection of 50 µl PBS or 50 µl chemokine diluted in PBS (= 100 ng), and 3 h after footpad injection of 50 µg (50 µl) pLacz or pcDNA3, very low endotoxin (<5 EU).
Same injections ate Day 0,7,14,21. Blood sampling for serum at Day 0,14,28.
Day 28: scarifice of all mice (groups of 6 female BALB/c mice, 6-week old at the beginning of the protocole). Recovery of spleen and popliteal lymph nodes. Single suspension and red blood-cell depletion for spleen.
Cell counts

	Polpiteal lymph node		spleen	
	Total x 10^{-6}	/animal x 10^{-6}	Total x 10^{-6}	/animal x 10^{-6}
PcDNA3	9.66	1.61	265	44.17
PBS + pLacZ	18.96	3.16	285	47.50
MIP-3α + pLacZ	21.6	3.6	250	41.67
MCP-4 + pLacZ	33	5.50	325	54.17
TECK + pLacZ	22.8	3.8	300	50.00

Culture set up in clone medium + 20 ng IL-2 + 1µM BP-1 with /ml: 5 x 10^6 spleen cells (24 well/group) or 2 x 10^6 lymph node cells (all cells put in culture).
Day 3: add 1 ml medium + IL-2 + BP-1

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Friday

Experiment irrelevant here

Monday

First experiment irrelevant here

→ ELISA for the adjuvant effect of hMCP-4, hMIP-3α (not dosed for TECK on that day because not enough coated plates) cf p164 for groups.
Standard protocole for the dosage of IgG, IgG1, IgG2a

- coat 5 µg/ml, 50 µl in PBS o/n @ 4°C
- 2 washes PBS
- block 2h @ 37°C
- pooled samples, 1/40 dilution than 1/2 to 1/2 dilutions, triplicates, 1 h @ RT
- anti Igs 1/1000 SAV-PAL 1h
- substrate 30 min
- OD 405-490.

EXHIBIT B, PAGE 2

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Raw data tables Day 0 and Day 14

A:pcDNA3 C: hMIP-3α + pLacz
B: pLacz (+PBS) D:hMCP-4 + pLacz
A1,2,3: dilutions 1/40, 1/80, 1/160 ...

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Raw data tables Day 28

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Final table